



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : B01L 1/00, G01N 33/543		A1	(11) International Publication Number: WO 00/16903 (43) International Publication Date: 30 March 2000 (30.03.00)		
(21) International Application Number: PCT/SE99/01658 (22) International Filing Date: 22 September 1999 (22.09.99) (30) Priority Data: 9803238-6 24 September 1998 (24.09.98) SE		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).			
(71) Applicant (for all designated States except US): BIOSENSOR APPLICATIONS SWEDEN AB (publ) [SE/SE]; Skäpplandsgatan 1, S-703 46 Örebro (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): GÅRDHAGEN, Peter [SE/SE]; Ringshyttans Gård, Striberg, S-713 93 Nora (SE). EDSTAM, Klas [SE/SE]; Backagårdsvägen 15, S-715 72 Stora Mellösa (SE). SAMUELSSON, Magnus [SE/SE]; Skogstorp, Ånsöga, S-633 49 Eskilstuna (SE). LANGEL, Ülo [SE/SE]; Trollesundsvägen 21, S-124 32 Bandhagen (SE). (74) Agents: NILSSON, Brita et al.; AB Stockholms Patentbyrå, Zacco & Bruhn, P.O. Box 23101, S-104 35 Stockholm (SE).		Published With international search report.			
(54) Title: BIOSENSOR CELL DEVICE AND ITS USE					
(57) Abstract					
<p>A biosensor cell device comprising a test chamber cavity (42) and a piezoelectric crystal (41) coated on the surface facing the test chamber cavity (42) with an interaction partner to an analyte, the crystal (41) being connected to an oscillation unit and signal processing electronics, is disclosed. The device further comprises an inlet needle (49) for the introduction of the analyte that ends at a small distance from the end of, and inside, an inlet sleeve (50) for the introduction of buffer solution that ends in the vicinity of the active surface of the crystal (41), the inlet sleeve being connected to an inlet (47) for buffer solution, and an outlet (48) for excessive fluid. The interaction partner may be an antibody which specifically binds to the analyte (antigen) e.g. a narcotic or an explosive such as 2,4,6-trinitrotoluene (TNT). The interaction partner may be a peptide nucleic acid (PNA) oligomer and the analyte may be a polynucleotide, such as RNA, DNA and PNA polymers complementary to the PNA oligomer, or vice versa. The biosensor cell device may be used for qualitative and quantitative analysis of at least one analyte and/or its interaction partner.</p>					

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

BIOSENSOR CELL DEVICE AND ITS USE

The present invention relates to a biosensor cell device and its use in qualitative and
5 quantitative analysis of at least one analyte and/or its interaction partner.

Background of the invention

In general, biosensors consist of two components : a highly specific recognition element and a transducer that converts the molecular recognition event into a quantifiable signal. The type of biosensor which is of interest in the present invention is a biosensor
10 operating with a piezoelectric crystal.

There are commercially available Quartz Crystal Analyzers, QCA, the systems which are based on analysis of the oscillation frequency changes connected to the changes in mass on the piezoelectric quartz crystal according to the following scheme.

15 Modified Piezoelectric
Quartz Crystal
and ⇒ Oscillation ⇒ Frequency ⇒ Analysis
Analyte Unit Counter of Data

20 A world-wide rising interest in the use of piezoelectric (PZ) crystals as analytical sensors, primarily as microbalances, has been demonstrated in the last years. The PZ crystal devices are used to determine the mass adsorbed on the active surface; the material on the surface of the PZ crystals, a monolayer, determines the specificity and selectivity of the sensor. By application of such microbalances or Quartz Crystal Analyzers, QCA., very high
25 sensitivity in analysis has been achieved. It is not unusual that picogram quantities of the analytes have been reported to be successfully detected.

Commercial prior art biosensor cell devices operate with the modified piezoelectric quartz crystal mounted in an open test chamber, i. e. with the analyte-containing liquid in contact with surrounding air. Therefore, change of analyte and/or modified crystal in these prior art QCA systems requires a fairly long time (3-5 minutes up to 2 hours or even more) before the system is stabilized and measurements can be made. (Cf. e.g. Wang J. et al, Mismatch-Sensitive Hybridizatin Detection by Peptide Nucleic Acids Immobilized on a Quartz Crystal Microbalance, Anal.Chem.1997,69,5200-5202).

It is evident that the application in routine analysis of available QCA systems is complicated due to the fact that they cannot be stabilized at short intervals, and further that available QCA systems are not suitable for portable detection systems.

Short description of the invention

5 The present invention is directed to a novel QCA device or system which is reliable, stable, quick, and sensitive. The novel device or system of the invention comprises a closed test chamber cavity which is filled with solvent/water or buffer solution, with or without analyte, and it is particularly suitable for applications where the direction of the test chamber may vary, such as in portable detection systems.

10 The biosensor cell device of the invention comprises a test chamber cavity and a piezoelectric crystal coated, on the surface facing the test chamber cavity, with an interaction partner to an analyte, the crystal being connected to an oscillation unit and signal processing electronics, which device further comprises an inlet needle for the introduction of the analyte that ends at a small distance from the end of, and inside, an inlet sleeve for the introduction of 15 buffer solution that ends in the vicinity of the active surface of the crystal, the inlet sleeve being connected to an inlet for buffer solution, and an outlet for excessive fluid.

The inlet needle may be an automatic injector or a syringe.

Further, the piezoelectric crystal may be coated with two or more different interaction partners each interacting with a different analyte.

20 In an example the interaction partner is an antibody which specifically binds to the analyte (antigen), e.g. selected from narcotics and explosives, such as 2,4,6-trinitrotoluene (TNT).

25 In another example the interaction partner is a peptide nucleic acid (PNA) oligomer and the analyte is a polynucleotide, or *vice versa*. The polynucleotide may be selected from the group consisting of RNA, DNA and PNA polymers complementary to the PNA oligomer.

The biosensor cell device according to the invention may be used for both qualitative and quantitative analysis of at least one analyte and/or its interaction partner.

SHORT DESCRIPTION OF THE DRAWINGS

Fig 1. shows a side view of the parts of a biosensor comprising a flow cell/test 30 chamber cavity.

Fig 2. shows partly a cross-section of the cell where the flow (arrows) is designed to pass over the surface of the crystal for both inlets of buffer and test samples.

Fig. 3. A : Analysis of 1 µg of 21-mer oligodeoxynucleotide complementary to 21-mer PNA.

B : Analysis of 1 µg of 21-mer oligodeoxynucleotide with scramble PNA 21-mer.

Fig. 4. Analysis of 1 µg of pRK8-hGaIR1 with complementary to hGaIR1 sequence 5 PNA 21-mer.

Fig. 5. Analysis of total RNA from human melanoma cell line Bowes with PNA 21-mer complementary to hGaIR1 cDNA.

A: 18 µg of RNA.

B: 80 µg of RNA.

10 **Fig. 6.** Analysis of dsDNA (348 µg) from human melanoma cell line Bowes with complementary to hGaIR1 PNA 21-mer.

DETAILED DESCRIPTION OF THE INVENTION

The biosensor cell device of the invention is used in the piezoelectric determination of an analyte, and it comprises a flow cell, in which the solvent/water or buffer solution, with or without 15 analyte, passes over the surface of an oscillating quartz crystal, coated with a layered structure exposing to the solution an interaction partner to an analyte, e.g. an antibody complex active against the analyte, such as TNT. The interaction partner interacts with the analyte, e.g. antibodies bind to the analyte, in the solution, and the weight difference, at the surface of the crystal, gives a slight change in the resonance frequency in the electronic circuit incorporating the crystal.

20 When the number of interaction partners, e.g. antibodies, available for interaction with or binding to the analyte is reduced due to the complex formation with the analyte in the test solution, the sensitivity will drop and the cell ceases to function. Then, the quartz crystal is replaced with another one which has a fresh coating.

In an automated embodiment, this situation will be detected by a signal processing unit, 25 and an automatic or manual change-over to a fresh flow cell takes place.

The drain pipe from one biosensor flow cell may be directly connected to the inlet of another, thus adding sensitivity to a different analyte, such as an explosive or a narcotic, or a multisensor system of parallel biosensors can be arranged by dividing the inlet into an array of flow cells.

30 The invention will now be illustrated with reference to the accompanying drawings, embodiments and examples. The invention is not intended to be limited to these specific disclosures.

The bottom part (40) is adapted to the design of the actual crystal (41). The crystal is kept in position by the upper part comprising the test chamber cavity (42), two rubber rings (43) and a sliding outer locking sleeve (44). The upper part of the cell is brought into its correct position by a steering pin (46) and two screws (45). The cell has an inlet for buffer solution (47), an outlet for excessive fluid (48) and a test sample inlet (49). The test sample inlet is shown with a syringe for manual injection.

The essential details of the biosensor cell device according to the invention are shown in **Fig. 1** and **Fig. 2**. The bottom part 40 is adapted to the design of an actual piezoelectric crystal 41. The crystal is kept in position by the upper part comprising a test chamber cavity 42, two rubber rings 43 and a sliding outer locking sleeve 44. The upper part of the cell is brought into its correct position by a steering pin 46 and two screws 45. The cell has an inlet for buffer solution 47, an outlet for excessive fluid 48 and a test sample inlet 49. The test sample inlet is shown with a syringe for manual injection.

The piezoelectric crystal 41 is coated on the surface facing the test chamber cavity 42 with an interaction partner to an analyte, and the crystal 41 is connected to an oscillation unit and signal processing electronics. The inlet needle 49 for the introduction of the analyte ends at a small distance from the end of, and inside, an inlet sleeve 50 for the introduction of buffer solution that ends in the vicinity of the active surface of the crystal 41. The inlet sleeve is connected to an inlet 47 for buffer solution. The device also comprises an outlet 48 for excessive fluid.

In preferred embodiments of the biosensor cell device of the invention the inlet needle 49 is an automatic injector or a syringe.

Examples of different embodiments of the invention are biosensor cell devices wherein the piezoelectric crystal 41 is coated with two or more different interaction partners each interacting with a different analyte; the interaction partner is an antibody which specifically binds to the analyte (antigen); the analyte(s) is (are) selected from narcotics and explosives; the explosive is 2,4,6-trinitrotoluene (TNT); the interaction partner is a peptide nucleic acid (PNA) oligomer and the analyte is a polynucleotide; the interaction partner is a polynucleotide and the analyte is a peptide nucleic acid (PNA) oligomer; and, the polynucleotide is selected from the group consisting of RNA, DNA and PNA polymers complementary to the PNA oligomer.

An other aspect of the biosensor cell device according to the invention is directed to its use for qualitative and quantitative analysis of at least one analyte and/or its interaction partner.

Examples of Analytes

As the detection targets of the biosensor cell device of the invention two classes of analytes, namely explosives and polynucleotides, have been chosen as preferred embodiments. These two large classes of analytes in turn consist of many different substances.

- 5 The detection of these with high sensitivity is of enormous importance today, both in the world welfare (explosives) and in biomedical analysis (polynucleotides).

TNT analysis is achieved by application of antibodies specific for TNT attached to the PZ gold(Au) electrodes.

- Analysis of polynucleotides is based on binding of RNA or DNA polymers to 10 specifically PNA modified PZ electrodes followed by QCA detection. RNA/DNA polymers are extracted from human tissue samples and applied to the Cys-PNA-electrode. When the mass increases the frequency of resonance oscillation of the PZ electrode decreases. The results are obtained in form of a diagram where the time is a function of the frequency. The 15 detection of the interaction is obtained in few seconds. Here we demonstrate the detection of polynucleotides and their interactions with biosensor QCA method where the interaction between relatively short (18- to 21-mers) peptide nucleic acids, PNA, and any complementary polynucleotides is applied. The PNA-PNA, PNA-DNA and PNA-RNA interactions are detected with piezoelectric (PZ) crystals as analytical sensors in the Quartz Crystal Analysis, QCA, system.

- 20 *Trinitrotoluene, TNT, as analyte.*

The antibodies recognizing TNT are used in the Examples of analyses using the biosensor cell device of the invention for the detection of picograms of a small antigen like TNT.

Polynucleotides as analytes or interaction partners to analytes

- Detection of polynucleotides as well as their interactions is crucial for biomedical 25 analysis and drug analysis as well as for research. The broader goal of the detection of the presence and interactions between polynucleotides with PNA oligomers is to detect various genetically linked disorders. It is well known that many serious disorders such as tuberculosis, malaria, Alzheimer's disease, HIV, cancer, cystic fibrosis etc. are connected to expression of mutated genes or overexpression of certain genes yielding in overexpression of 30 characteristic proteins. In case of occurrence of disorder-linked mutations or overexpression in the case of patients, those will be detected by interaction with complementary to the mutations or overexpressed product PNA oligomers coupled to the PZ crystals. The application of short PNA polymers complementary to disease- associated mutant genes will enable the analysis of any mutation of interest by simple design and synthesis of the short

complementary PNA sequence. Such a method of analysis will be very flexible and cheap as compared to the methods available today, especially by use of the rapid and sensitive QCA system of the present invention.

Peptide nucleic acids, PNA, as analytes or interaction partners to analytes.

Recently, peptide nucleic acids (PNA) were introduced by Nielsen et al.(Nielsen P.E., Egholm, M., Berg, R.H., and Buchardt, O. 1991, Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* 254:1497-1500) as a replacement of the entire ribose-phosphodiester backbone in oligonucleotides with N-(2-aminoethyl)glycine units, to which appropriate nucleobases are attached via carbonyl methylene linkers. The discovery of PNAs has given the possibility of radically different synthetic versions of the DNA and RNA. The advantages of this chemical strategy are many. The PNA monomers can be polymerized using standard peptide coupling chemistry, which is flexible, highly developed and efficient. The hybrids between such PNA oligomers and their complementary single stranded DNA oligomers have shown remarkably high stability (affinity) in comparison with naturally occurring DNA/DNA and RNA/DNA hybrids. Thus, PNA oligomers offer great advantages in both antisense and antigen approaches for regulating gene expression. Moreover, the PNA oligomers are considerably more stable towards nucleases (compared to natural polynucleotides) due to the lack of (deoxy)ribose backbone.

These properties of PNA can be efficiently applied for any approach requiring non-degrading high affinity polymers for RNA/DNA recognition. Hybridization of PNA oligonucleotides to complementary RNA/DNA sequences by specific base-pairing (A-T and G-C) is caused by interaction through hydrogen bonding. This simple base-pairing allows the design of PNA oligonucleotides that target any gene or RNA of a known sequence. A major advantage of this strategy is in the potential specificity of action. High specificity and affinity of the interactions lead to more sensitive detection methods in DNA/RNA estimations.

Oscillation unit, signal processing electronics, and electrodes

The crystal in the biosensor is excited in a modified Pierce oscillator. The standard Pierce oscillator is modified with:

- 30 - an extra buffer stage in the oscillating loop to compensate for the high damping of the crystal by the fluid,
- a DC blocking capacitor to eliminate electrolysis by stray currents through the buffer fluid system,

- a buffer stage and a signal transformer to galvanically separate the measurement circuits from the frequency counter and analyze computer.

The signal processing electronics contains the electronics for driving the crystal, measurement of different parameters and decision algorithms for evaluation of the results.

- 5 The electronics for driving the crystal and measure the frequency shift consists of an oscillator (Colpitt or Pierce style) with the crystal followed by a buffer amplifier and a fast precision counter with the interpolation. The results from the frequency measurements are fed to the control computer for evaluation.

10 The electrodes used in the Examples are AT-cut 9 - 10 MHz piezoelectric crystals with gold electrodes, supplied by Quartz Probe, Sweden, or Seiko, Japan.

EXAMPLES OF ANALYSES USING THE BIOSENSOR CELL DEVICE

Reagents and biochemical methods

General reagents

B1 : Na-HEPES buffer: 0.01 M, pH 7.4.

15 HEPES : Fluka 54466, MW 260.30; adjust pH with 0.01 M HCl

B2 : 0.01 M Na-phosphate buffer, 0.1 M NaCl, pH 7.4, consists of Na₂HPO₄.12H₂O: 3.58 g/l;

NaH₂PO₄.H₂O : 1.38 g/l ;

NaCl 5.85 g/l. Adjust pH with the salts

Water: Deionized tap water

20 Thiol or disulphide containing modification reagents can be used with Au-electrode

TNT analysis

Electrode-bound TNT-antibodies were used. TNT-antibodies are commercially available.

Polynucleotide analysis

1. Reagents

25 PNA monomers : commercial, PerSeptive Biosystems, Framingham, USA

Peptide synthesis reagents : described in Design of chimeric peptide ligands to galanin receptors and substance P receptors. Int. J. Pept. Protein Res. 39, 516-522. (1992) Langel, Ülo., Land, T., and Bartfai, T.

2. Covalent immobilization of the Cys-PNA oligomers to the electrodes. Preparation of the electrode for measurement

The ethanol-washed and dry electrode was incubated in the 1 µM solution of the appropriate Cys-PNA 21-mer solution in B2 for 14-16 h at room temperature in dark and in tightly closed tube. The electrode was washed with water, incubated for 5 min in water.

The PNA-modified electrode was placed into the cell and washed with B2, 100 ml/h, 10 ml, the electrode was stabilized (change is less than 2 Hz/10 min) for 20 - 60 min.

3. Analysis of polynucleotides

30-50 µl of diluted analyte (21-mers of oligonucleotides, plasmids, RNA or DNA from cells) was injected into the cell with PNA-modified and prepared electrode, the change of frequency was registered.

4. PNA synthesis

Synthesis of PNA polymers was performed manually as described in using *t*-Boc chemistry and monomers from PerSeptive Biosystems (Framingham, MA, USA). Thiol moiety was introduced into PNA polymer as amino acid Cys in 5' position of PNA oligomers.

Sequences of PNA oligomers are presented in the accordance with the peptide nomenclature (the N-terminus of the PNA oligomer lies on left).

21-mer cPNA, complementary to region 18-38 of the human galanin receptor type 1:
GC GTT GCC CTC GCT GAG GTT C amide

21 -mer scrambled PNA: GGC ATG GCT GCT CTC CGT CTG amide

1 -mer cPNA, complementary to region 18-38 of the rat galanin receptor type 1: CC
ATT CCC TTC ACT GAG GTT C amide

5. Separation of DNA

Cells were resuspended in 1 vol of digestion buffer. The samples were incubated with shaking at 50°C for 12 to 18 h and extracted with equal volume of phenol/chloroform/isopropanol followed by centrifugation for 10 min at 1 700 x g in a swinging bucket rotor.

The aqueous (top) layer was transferred to a new tube and 1/2 vol of 7.5 M ammonium acetate and 2 vol of 100% ethanol were added. The pellet was rinsed with 75% ethanol, ethanol was decanted and the pellet was dried. DNA was resuspended in TE buffer until dissolved.

6. Separation of RNA

Cells were lysed by adding UltraspecTM RNA. 0.2 ml of chloroform per 1 ml of UltraspecTM RNA was added and shaken at 4°C for 5 min. The homogenate was centrifuged at 12 000 x g (4°C) for 15 min. The aqueous phase was carefully transferred and equal volume of isopropanol was added; the samples were incubated for 10 min at 4°C. The supernatant was removed and the RNA pellet was washed twice with 75% ethanol by vortexing and subsequently centrifuged for 5 min at 7 500 x g at 4°C. The pellet was dried under vacuum for

5-10 min. The RNA pellet was dissolved in 50-100 µl Ultraspec™ DEPC treated water.

7. Plasmid

A mammalian vector pRK8, derived from vector pRK5, was applied as a hGalR1 cDNA sequence containing construct. The human galanin receptor cDNA, hGal R1, from the 5 human melanoma Bowes cell line was PCR-cloned and inserted into the EcoR1 and Not1 cleaved pRK8. The inserted receptor which has 21-mer homology region with PNA probe, was used in preliminary experiments to demonstrate specificity and selectivity of PNA biosensor by interacting with PNA electrode.

8. Oligonucleotides

10 Sequences of complementary DNA oligomers GIBCOBRL, B1958B11, MW 6834 µg/µmol were applied to the 21-mer homology region with 21-mer cPNA complementary to region 18-38 of the rat galanin receptor type 1.

Sequence of 21-mer CR DNA: GAACCTCAGTGAAGGGAATGG

Sequence of 21-mer cPNA: CTTGGAGTCACTTCCCTTACC

15 9. Regeneration of the electrode for further application

Regeneration of the electrode is achieved by adding RNases or DNases which degrade the specifically bound RNA or DNA, respectively. Another approach to prepare the once used PNA-electrode for the next application is to wash the specifically bound polynucleotides by washing of the cell with the buffers with different ionic strength and water.

20 10. Kinetics of duplex or triplex formation

The equilibrium of binding of oligonucleotides to PNA-electrodes is achieved within 100 - 200 s, the time which is convenient for determination of the interaction kinetics without additional equipment. Hence, the method is applicable for measurement of the kinetics of the interaction between the PNA and analyzed RNA or DNA monomers or dimers.

25 11. Immobilization of biotinyl-PNA polymer to streptavidin coated electrode

It is possible to simply couple biotinyl moiety into the appropriate sequence of the PNA polymer. It is possible as well to coat the QCA electrode with avidin or streptavidin, and to use the coated electrode to bind the biotinyl-PNA. This method is alternative to the coupling of PNA polymers to the electrodes by reaction of SH-groups with gold surface (cf. above).

30 12. Cell cultures

Rat Rin m5F cells were cultivated in RPMI 1640 (Gibco 041-01870), supplement with 5% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml

streptomycin. The cells were grown over 3 days to about 50 % confluence in tissue culture flasks.

Rat SHSY cells were cultivated in Minimal Essential Medium with Earles salts (MEM), supplement with 10 % fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 5 100 µg/ml streptomycin, 1 % non essential amino acids. The cells were grown over 3 days to about 50% confluence in tissue culture flasks.

RESULTS

A. TNT analysis

Application of piezoelectric (PZ) crystals as analytical sensors, in Quartz Crystal Microbalance (QCM), has been described in our co-pending patent application 10 PCT/EP98/03531, the contents of which is incorporated herein by reference, in detection of the small amounts of TNT in air probes collected from ground presumably containing mines followed by concentration.

In the general method in these studies, the 9-10 MHz, electrode, QA-A9M-Au, 15 Seiko, or Quartz Probe, Sweden carrying electrode-bound TNT-antibodies, is used. The change in frequency of the electrode due to antibody-antigen interaction is registered in time.

Operation of QCA with commercial measuring unit is very sensitive for electrical and physical disturbances, e.g. it is recommended not to use radio in the same room with the QCA; the pump vibrations disturb the measurements significantly. It is highly recommended 20 therefore that the conditions of the measurements will be well defined. The stability of the system varies from day to day and even from electrode to electrode, sometimes even different sides of the electrodes behave differently.

In 3 different experiments we have used the same electrode (both sides) in both a commercial system and in the system of the invention in the following way. The effect of 25 TNT was measured in a commercial, Seiko's, system first and no effect of TNT was obtained. Another side of the electrode was used in the system of the invention and the effect of TNT was obtained. In 2 different experiments we have used the same electrode (both sides) in both systems in the opposite way. The effect of TNT was measured in the system of the invention first and the effect of TNT was obtained. Another side of the electrode was used in Seiko's 30 system and the effect of TNT was not obtained. The Seiko's electronics could heat up the electrode during the measurements and, hence, cause the disappearance of the effect of TNT.

The construction of the biosensor cell of the invention permits the stabilization of the system in 1-2 min or faster. The method of TNT detection by application of specific antibodies on QCA resulted in detection of TNT at the low level of 10 pg TNT.

B. Polynucleotide analysis

5. The initial analogs of PNAs belong to the complementary human and rat brain galanin receptor cDNA-derived sequences (cf. above). The 21-mers from human galanin receptor cDNA serve as lead sequences due to their demonstrated high specificity in preliminary experiments.

In preliminary experiments, these 21-mers of PNA were coupled to PZ electrodes, 10 the coupling was followed by the change of 500 - 1000 Hz in frequency. The modified electrodes were then exposed to the following sources of oligonucleotides and polynucleotides.

1. Complementary DNA 21-mers, sequences cf. above.
2. Plasmid with complementary DNA regions included.
- 15 3. Total cellular RNA from cell lines expressing respective galanin receptors.
4. Double strand DNA from cell lines expressing respective galanin receptors.

By registering the change in frequency, the interaction of immobilized PNA with cellular RNA or DNA was registered. The results are presented in Fig. 3 through Fig. 6. As a control injection in each analysis, 30-50 µl of buffer solution was injected.

20. As negative controls, two different approaches were applied.

1. Electrodes immobilized with rat and human PNA 21-mers were exposed to RNA from cell lines not expressing the galanin receptors, SHSY.
2. An electrode immobilized scramble PNA 21-mer sequence (as compared to complementary sequences) was exposed to galanin receptor expressing cellular RNA and DNA.

25. In each case, a change in frequency was not registered in control analysis.

Sensitivity

The data about the analysis of DNA, RNA, DNA 21-mers and plasmids are presented in Table 1. One can see that relatively small amounts of the polynucleotides could be analyzed. The amount of 30-50 ng of 21-mer DNA, 160-200 µg plasmid, 1 µg of RNA and 30 12-18 µg of DNA were efficiently analyzed by PNA-QCA method. The lower limit in amount for RNA and plasmid analysis could be even lower, however, this analysis has not been carried out yet.

Chemical modification of Au-PZ electrodes with Cys-PNAs, in order to analyze by QCA specifically binding RNA or DNA polymers extracted from human tissue samples, is a relatively simple method. The PZ 9-10 MHz electrodes are commercially available. The QCA equipment presented in this application is much more sensitive and reliable as compared to
5 the commercial systems.

Table 1. Analysis by PNA-QCA of different amounts of different polynucleotides

Interaction of PNA with	Change of frequency (Higher concentration)	Change of frequency (Lower concentration)
Total DNA	-103 Hz (18 µg) -230 Hz (12 µg)	-8 Hz (1.2 µg)
Total RNA	-134 Hz (3.2 µg) -69 Hz (3.2 µg)	-37 Hz (1 µg)
21-mer oligonucleotide	-17 Hz (30 ng) -13 Hz (50 ng)	-2 Hz (15 ng) -1 Hz (10 ng)
Plasmid	-36 Hz (200 µg) -34 Hz (160 µg)	-31 Hz (58 µg)

Abbreviations

- 5 *t*-Boc : *tert*-butyloxycarbonyl
 cDNA: complementary DNA
 DEPC : diethylpyrocarbonate
 DNA: deoxyribonucleic acid
 DNT: dinitrotoluene
- 10 EcoR1 : a restriction endonuclease from *E. coli* RY13
 hGal R1 : human galanin receptor type 1
 Not1 : a restriction endonuclease from *Nocardia otitidis-cavaeum*
 PCR: polymerase chain reaction
 PNA: peptide nucleic acid
- 15 pRK8: mammalian vector derived from vector pRK5
 PZ : piezoelectric
 QCA : quartz crystal analysis
 RNA: ribonucleic acid
 TE: tris-EDTA
- 20 TNT: trinitrotoluene
 UltraspecTM : DNA and RNA isolation reagent, contents : phenol, guanidine salts, urea,
 buffering agents, detergent and stabilizer.
-

Claims

1. Biosensor cell device comprising a test chamber cavity (42) and a piezoelectric crystal (41) coated on the surface facing the test chamber cavity (42) with an interaction partner to an analyte, the crystal (41) being connected to an oscillation unit and signal processing electronics, characterized in that the device further comprises an inlet needle (49) for the introduction of the analyte that ends at a small distance from the end of, and inside, an inlet sleeve (50) for the introduction of buffer solution that ends in the vicinity of the active surface of the crystal (41), the inlet sleeve being connected to an inlet (47) for buffer solution, and an outlet (48) for excessive fluid.

2. Biosensor cell device according to claim 1, wherein the inlet needle (49) is an automatic injector.

3. Biosensor cell device according to claim 1, wherein the inlet needle (49) is a syringe.

4. Biosensor cell device according to any one of claims 1 - 3, wherein the piezoelectric crystal (41) is coated with two or more different interaction partners each interacting with a different analyte.

5. Biosensor cell device according to any one of claims 1 - 3, wherein the interaction partner is an antibody which specifically binds to the analyte (antigen).

6. Biosensor cell device according to claim 4 or 5, wherein the analyte(s) is (are) selected from narcotics and explosives.

7. Biosensor cell device according to claim 6, wherein the explosive is 2,4,6-trinitrotoluene (TNT).

8. Biosensor cell device according to any one of claims 1 - 4, wherein the interaction partner is a peptide nucleic acid (PNA) oligomer and the analyte is a polynucleotide.

9. Biosensor cell device according to any one of claims 1 - 4, wherein the interaction partner is a polynucleotide and the analyte is a peptide nucleic acid (PNA) oligomer.

10. Biosensor cell device according to claim 8 or 9, wherein the polynucleotide is selected from the group consisting of RNA, DNA and PNA polymers complementary to the PNA oligomer.

11. Use of a biosensor cell device according to any one of claims 1-8 for qualitative and quantitative analysis of at least one analyte and/or its interaction partner.

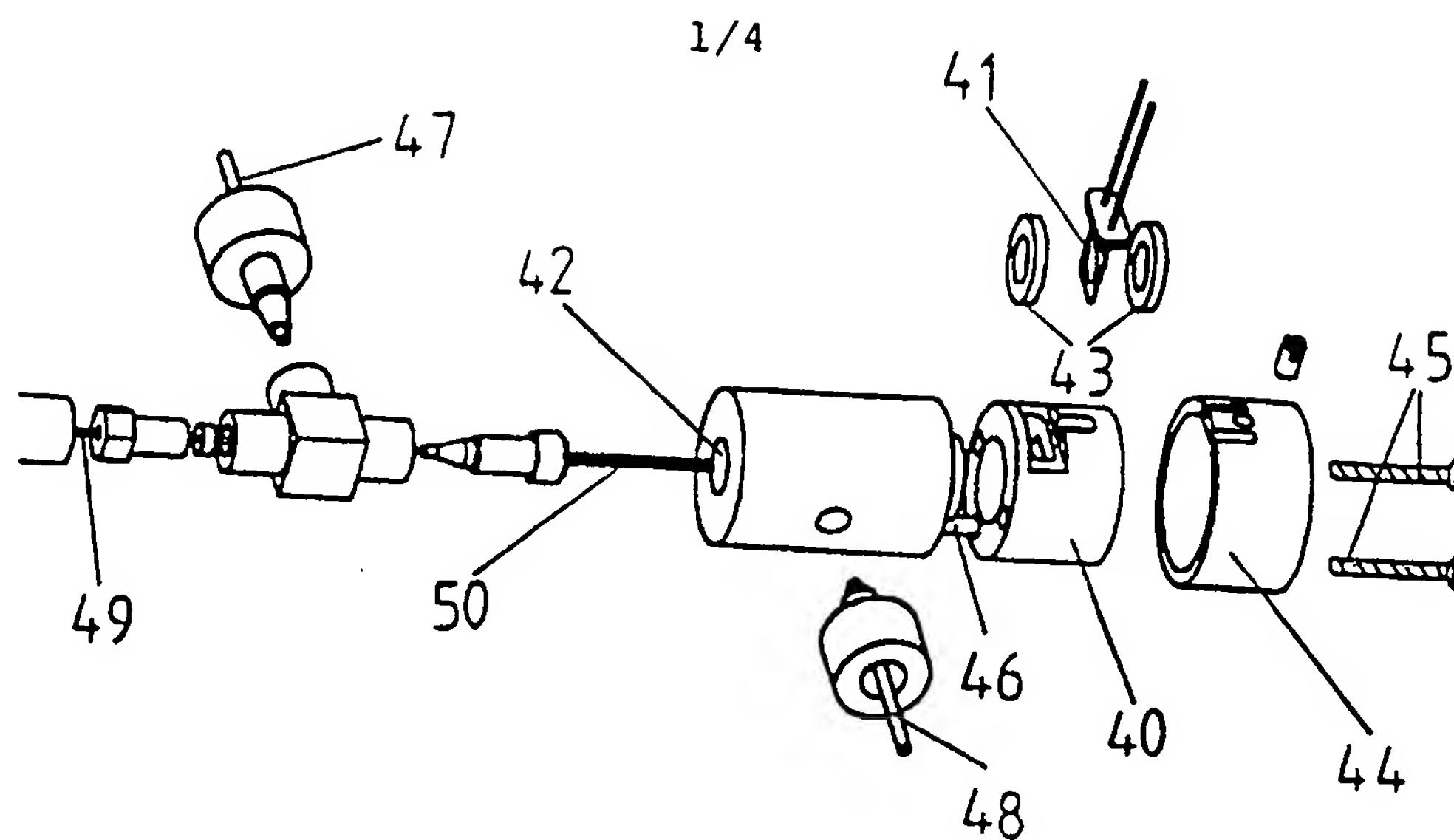


Fig. 1

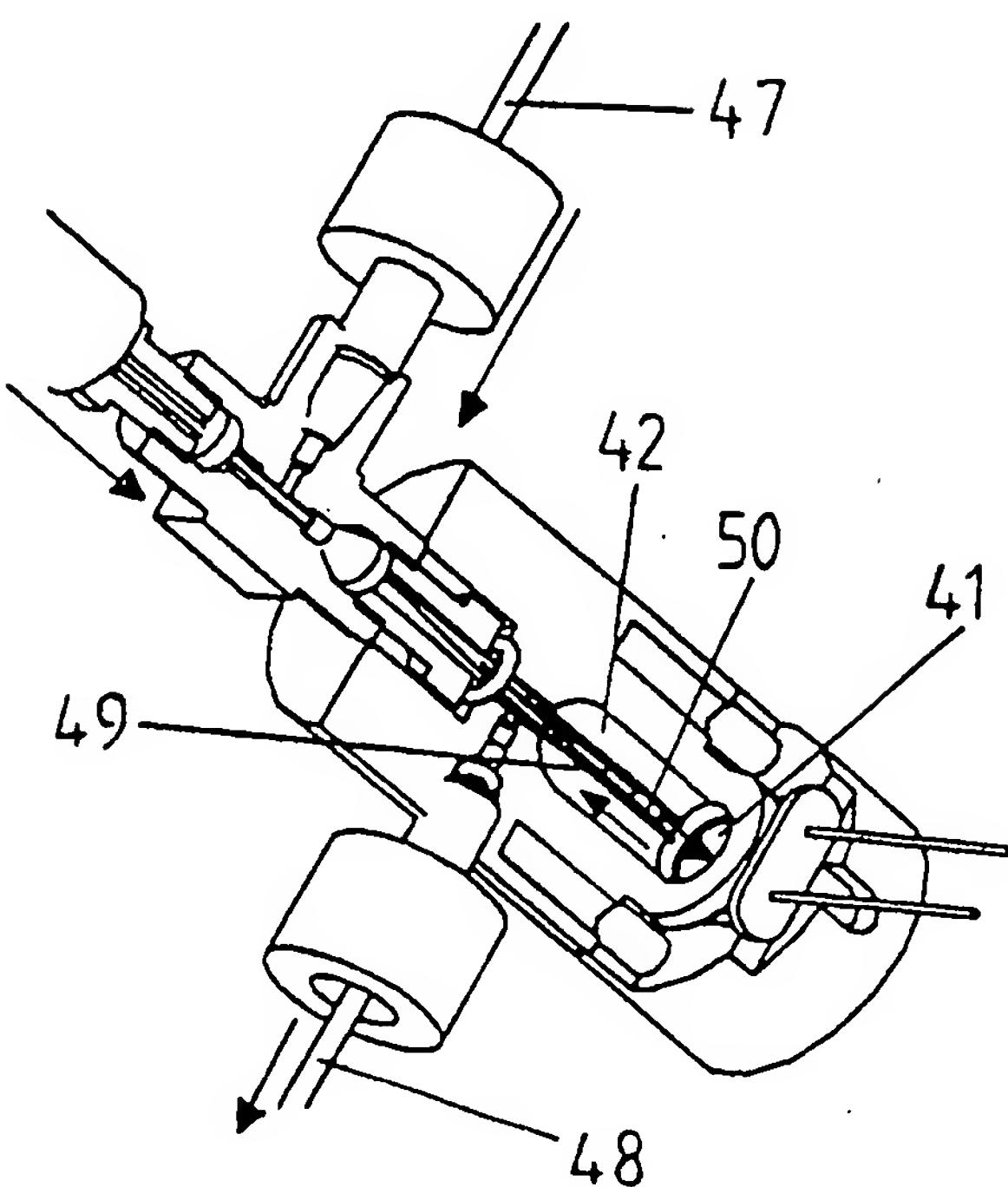
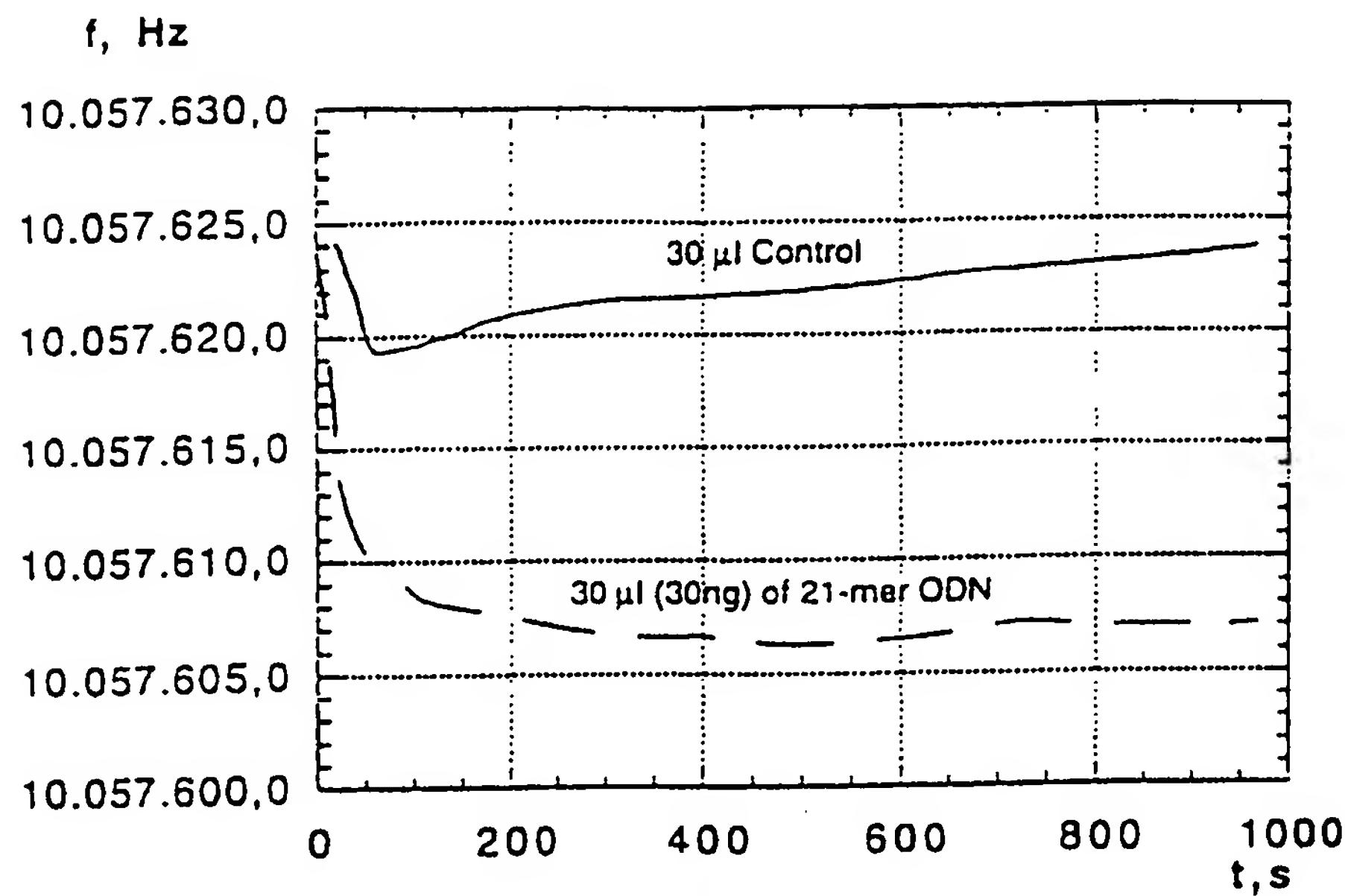
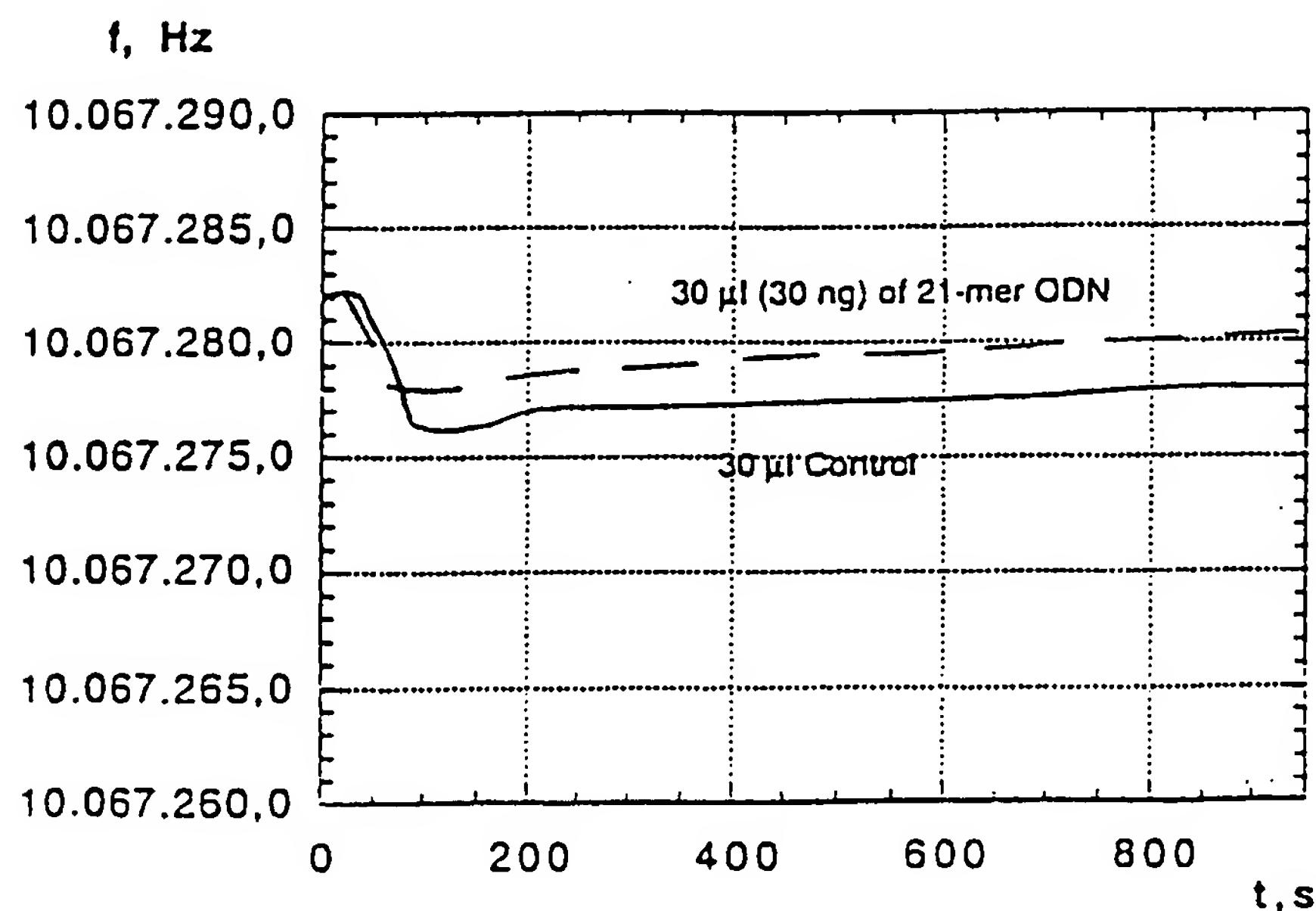


Fig. 2

2/4

**Fig. 3A****Fig. 3B**

3/4

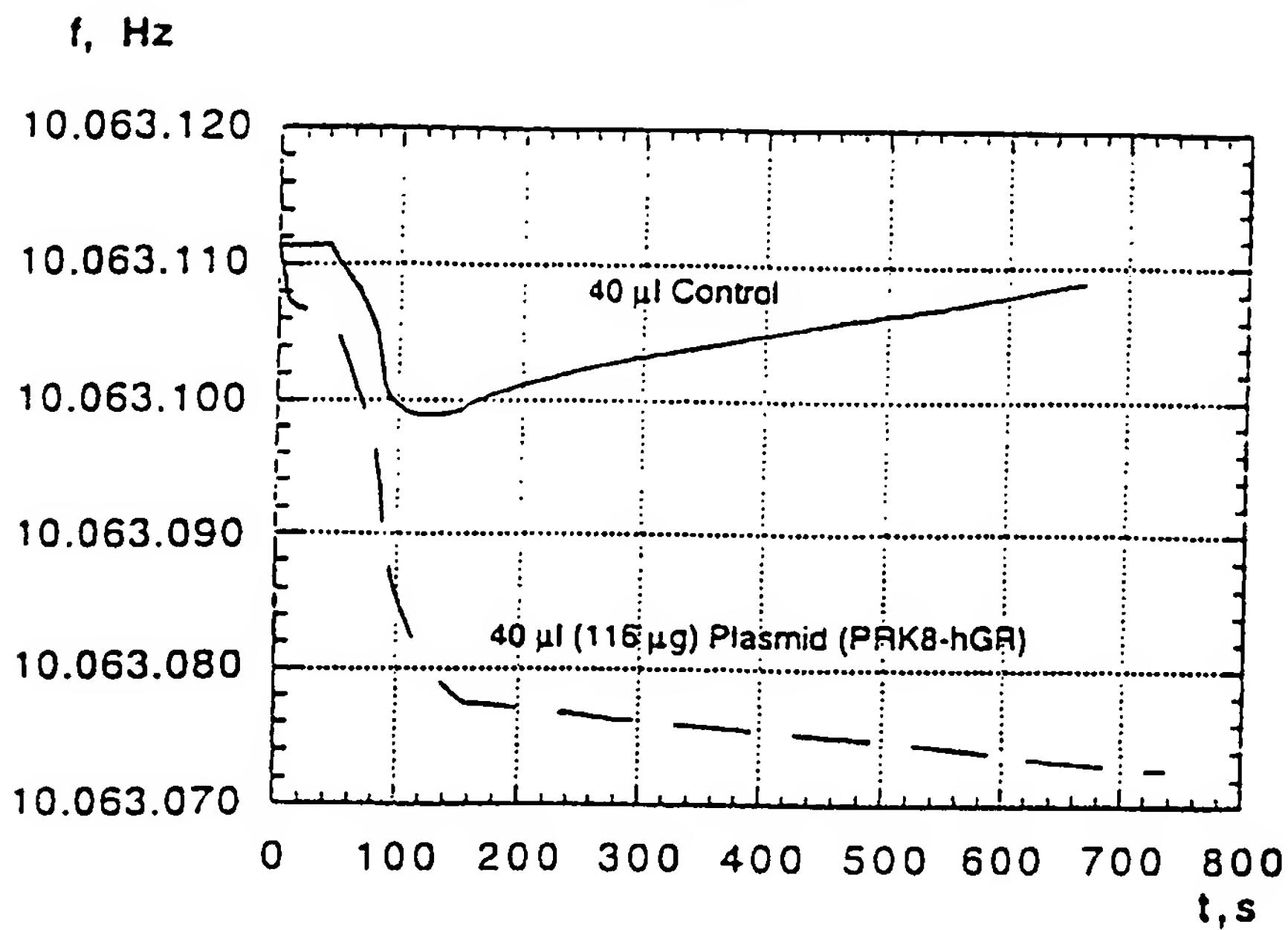


Fig. 4

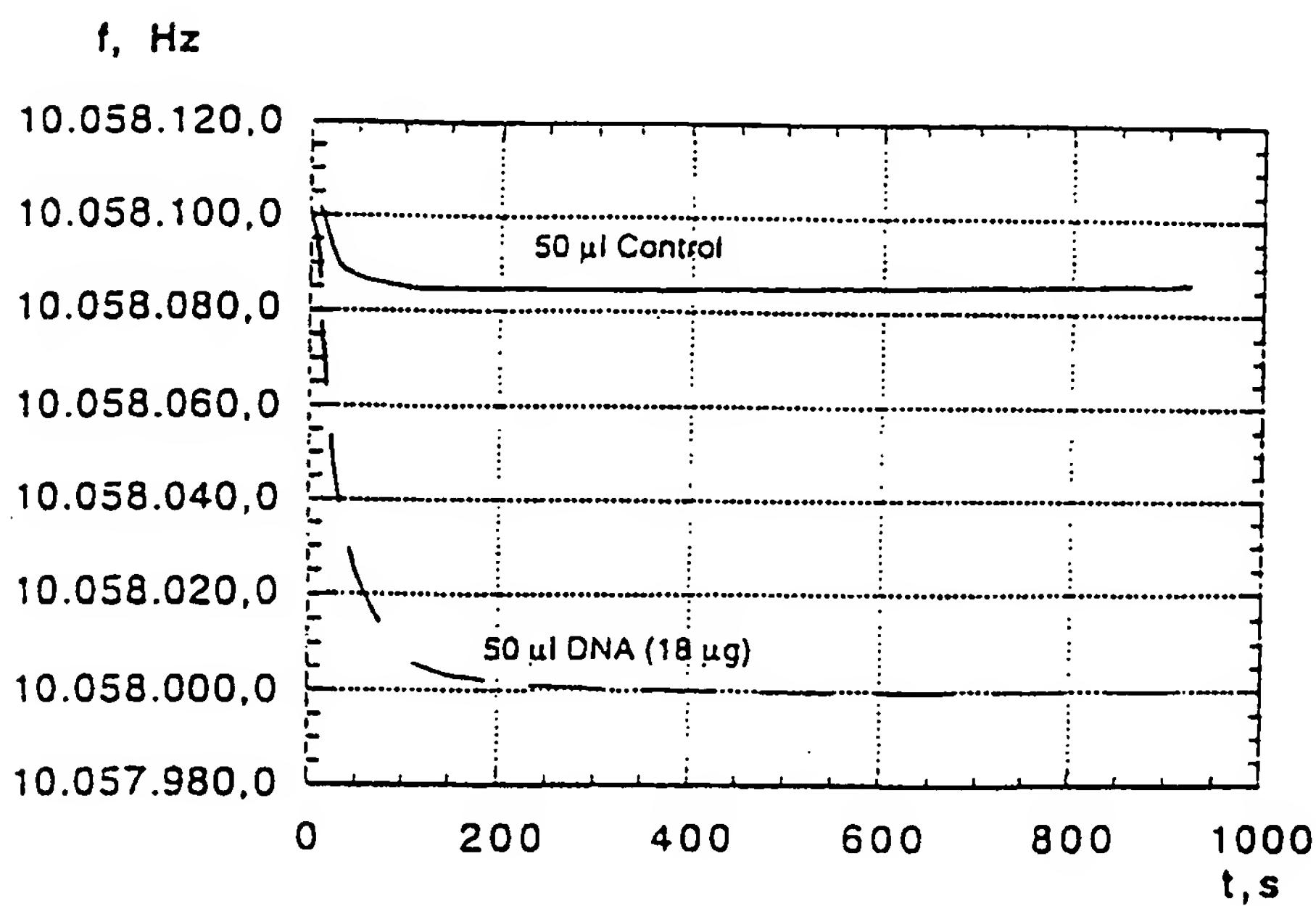


Fig. 6

4/4

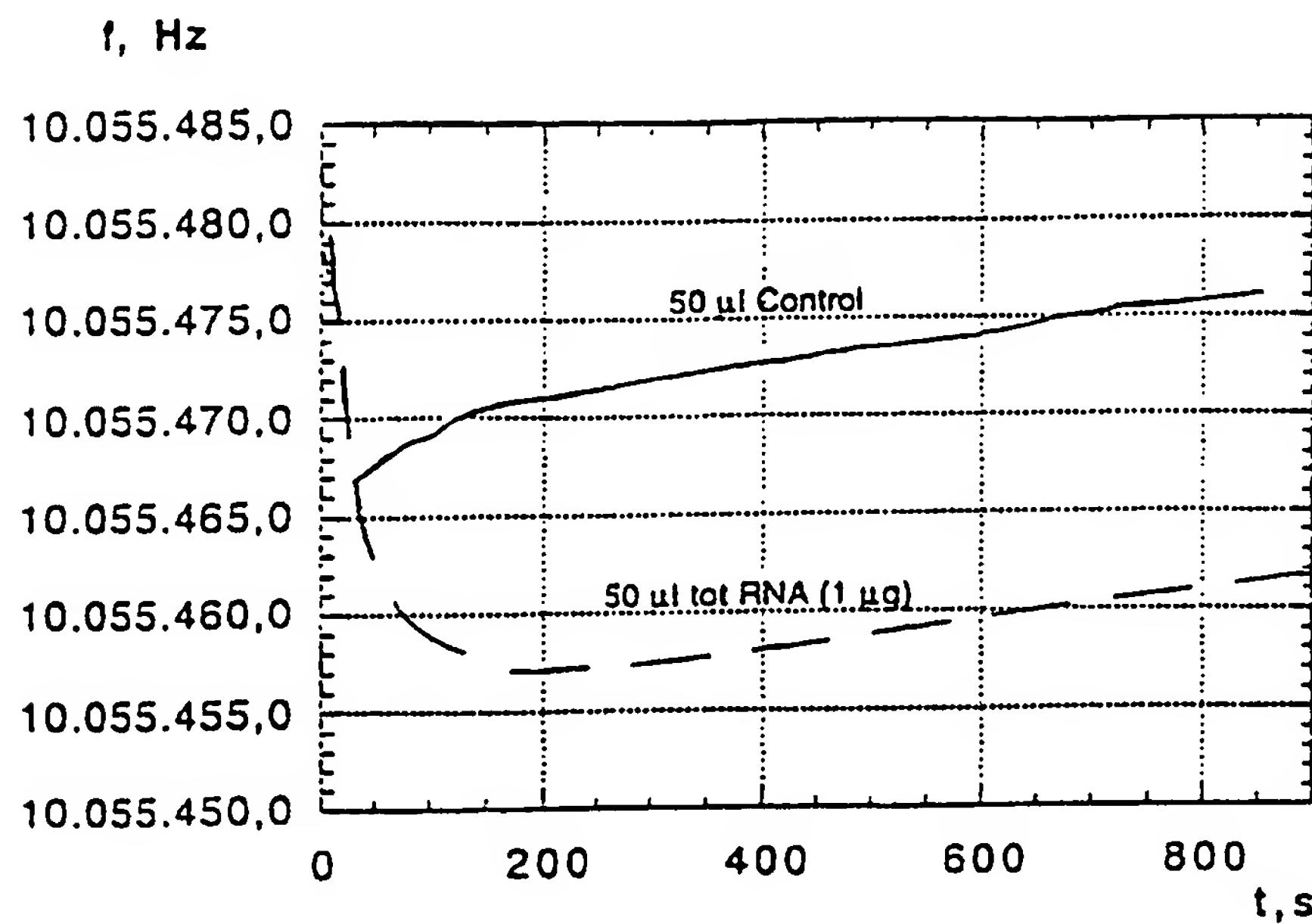


Fig. 5A

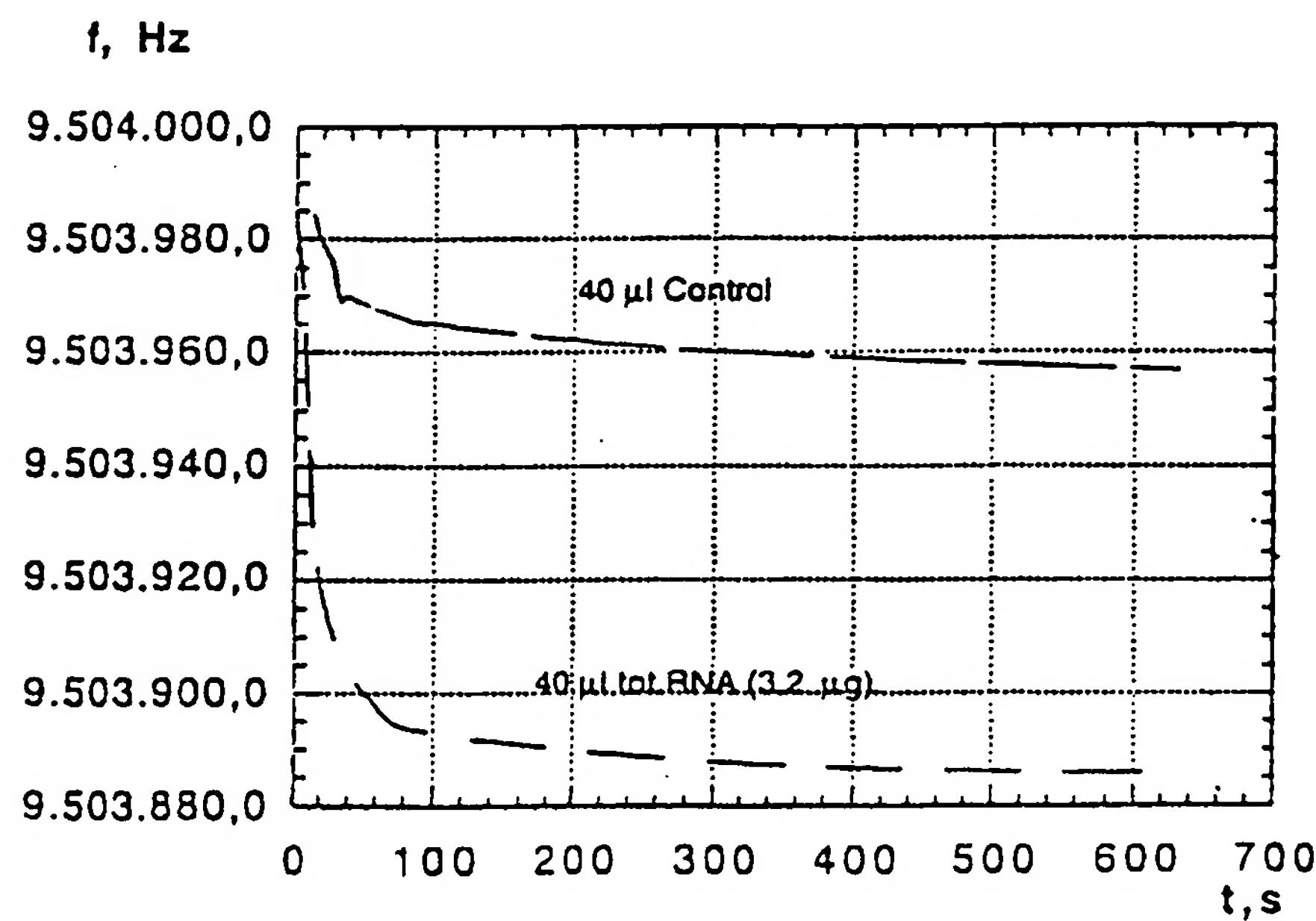


Fig. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01658

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B01L 1/00, G01N 33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: B01L, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9828623 A1 (GAMERA BIOSCIENCE CORPORATION), 2 July 1998 (02.07.98), See esp fig 2 --	1-11
A	US 5468605 A (ALBERT K.M. HARRIS ET AL), 21 November 1995 (21.11.95), See abstract and figure 2 --	1-11
A	WO 9704314 A2 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HERBREW UNIVERSITY OF JERUSALEM), 6 February 1997 (06.02.97), See abstract and claims --	1-11

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"J" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 January 2000

Date of mailing of the international search report

12-01-2000

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer
Hampus Rystedt/MN
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01658

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>Analytical Chemistry, Volume 69, No 24, December 1997, Joseph Wang et al, "Mismatch-Sensitive Hybridization Detection by Peptide Nucleic Acids Immobilized on a Quartz Crystal Microbalance" page 5200 - page 5202</p> <p>--</p>	1-11
A	<p>EP 0781999 A1 (BEHRINGWERKE AG), 2 July 1997 (02.07.97)</p> <p>--</p> <p>-----</p>	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.

PCT/SE 99/01658

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9828623 A1	02/07/98	AU 5895898 A		17/07/98
US 5468605 A	21/11/95	NONE		
WO 9704314 A2	06/02/97	AU 6317896 A EP 0843816 A IL 114692 D JP 11510247 T		18/02/97 27/05/98 00/00/00 07/09/99
EP 0781999 A1	02/07/97	AU 7549896 A CA 2193318 A DE 19548376 A JP 9189701 A		03/07/97 28/06/97 03/07/97 22/07/97